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Abstract
Pterins accumulate in the head capsules of horn flies, *Haematobia irritans irritans* (L.), as a linear function of time and temperature. Pterin concentrations were used to estimate chronological ages and to establish correlations between chronological age and ovarian development and reproductive success in 12 horn fly populations in 1988 and 1989. Male ages were estimated spectrofluorometrically. There were statistically significant differences between years in population age structure measured by pterins. Survival rates estimated from pterin concentration distributions were consistent with a one-parameter exponential model with constant survival rate. Mean daily survival rates were 0.81 for females and 0.84 for males in 1988 and 0.66 and 0.75 in 1989. Mean lifetime egg production was ≈26 eggs per female in 1988 and 8 in 1989. Female reproductive success was close to the maximum possible, i.e., there were no net delays in oviposition. Analysis of gonotrophic age distributions provided survival estimates that suggested an increasing risk of mortality with age or age-related biases in sampling.

Keywords
Insecta, pterins, Haematobia irritans irritans, population age structure

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Age Structure of Horn Fly (Diptera: Muscidae) Populations Estimated by Pterin Concentrations

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ABSTRACT Pterins accumulate in the head capsules of horn flies, *Haematobia irritans irritans* (L.), as a linear function of time and temperature. Pterin concentrations were used to estimate chronological ages and to establish correlations between chronological age and ovarian development and reproductive success in 12 horn fly populations in 1988 and 1989. Male ages were estimated spectrofluorometrically. There were statistically significant differences between years in population age structure measured by pterins. Survival rates estimated from pterin concentration distributions were consistent with a one-parameter exponential model with constant survival rate. Mean daily survival rates were 0.81 for females and 0.84 for males in 1988 and 0.66 and 0.75 in 1989. Mean lifetime egg production was ≈26 eggs per female in 1988 and 8 in 1989. Female reproductive success was close to the maximum possible, i.e., there were no net delays in oviposition. Analysis of gonotrophic age distributions provided survival estimates that suggested an increasing risk of mortality with age or age-related biases in sampling.

KEY WORDS Insecta, pterins, *Haematobia irritans irritans*, population age structure

BIONOMICS OF HORN FLIES, *Haematobia irritans irritans* (L.), have been studied intensively in North America, but their demography is not well understood. An approximate schedule of feeding and oviposition can be inferred from the literature, and age grading of female reproductive systems affords estimates of reproductive success (Krafsur & Ernst 1983, 1986; Kuramochi & Nishijima 1984; Fay & Doube 1987). Also, age-grading methods have been applied to studies of dispersion (Guillot et al. 1988).

Only three reproductive age groups could be identified with confidence in Iowa horn flies (Krafsur & Ernst 1983), the previtellogenic (days 0, 1), vitellogenic (2 d and older), and parous (at least 3.5 d). The greatest error in determining parity was for gravid flies. Moreover, distinguishing multiparous from uniparous flies could not be done with confidence. An average mortality rate of 0.14 day^{-1} was estimated for Iowa horn flies on the chief assumptions that sampling was representative of all age groups, there were no delays in ovarian development and oviposition, and survival was independent of age (Krafsur & Ernst 1983).

The Iowa horn fly survival distributions were estimated from the proportions of vitellogenic (≥2 d of age) and parous (≥3.5 d of age) females. Both proportions were especially sensitive to the number of day-0 flies, the largest age group. A systematic sampling deficiency of this youngest age would lead to survival estimates that are too high. Identification of more age groups than previtellogenic, vitellogenic nullipars, and parous is required to evaluate the assumptions made in estimating horn fly age distributions.

An additional age-grading method now is available. The concentration of pterins (derivatives of 2-amino-4(3H)-oxopteridine [Ziegler & Harmsen 1969]) in the head capsules of *Stomoxys calcitrans* (L.) and *Glossina morsitans morsitans* Westwood were shown to accumulate linearly with age and temperature (Mail et al. 1983, Lehane & Mail 1985). A similar accumulation of pterins also occurred in the screwworm flies *Cochliomyia hominivorax* Coquerel (Thomas & Chen 1989) and *Chrysomya bezziana* Villeneuve (Wall et al. 1990).

The objectives of the present study were to use pterin concentrations as an age-grading tool independently of and in addition to estimates of gonotrophic age and to measure the age structure of replicate horn fly populations. Horn flies are especially suited for this research because the effects of temperature on ovarian development and rate of pterin deposition can be ignored. This is because the temperatures experienced by horn flies approximate the surface temperatures of their host cattle, ≈30°C, which is constant over time and most of the body surface (Kuramochi 1989). Moreover, flies will move if temperatures deviate from 30°C, for example, in strong sunlight.

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Materials and Methods

Correlations between pterin concentrations, ovarian development, and horn fly chronological ages were established for two cohorts indoors at the USDA Knipling–Bushland laboratory in Kerrville, Tex.; teneral flies were allowed to infest a clean steer, and adults were sampled at recorded intervals, placed in shell vials with $CaCl_2$ as a desiccant, and shipped on ice to Ames where pterin concentrations were measured and female reproductive systems examined. Ambient temperatures in the Kerrville laboratory varied from 17 to 27°C (mean, 23°C). Horn flies, however, move about on cattle probably in response to temperature (Morgan 1964), and prefer to congregate on body surfaces at ≈30°C (Kuramochi 1989). These flies are hereinafter referred to as "laboratory" flies.

Field Sampling. Horn flies were sampled by sweeping them from the backs and sides of cattle. Four herds in or near Ames were sampled in 1988. In 1989, there were five Ames herds, a herd from central Iowa, one from southern Iowa, and a herd from western Iowa. Herds were sampled in the morning once biweekly or monthly throughout the fly season. Cattle in 1988 were unconfined when samples were made, but six of eight herds were confined in chutes during sampling of flies in 1989. It is not established that horn flies distribute themselves on cattle randomly with regard to age or sex (Krafsur & Ernst 1986), and this raises the possibility of sampling biases.

The swept flies usually were confined alive in cages and maintained on ice in a large insulated box until they could be killed by freezing in the laboratory.

Age Grading and Size. Female horn flies were assigned to gonotrophic age classes as described by Krafsur & Ernst (1983). Head widths and mesonotal lengths were measured with an ocular micrometer to provide indices of body size. Follicle lengths were measured with an ocular micrometer, the degree of vitellogenesis was rated by using criteria of Tyndale-Biscoe & Hughes (1969), and the presence or absence of follicular relics were noted. Thorax (mesonotum) lengths and head widths were recorded for males.

Follicle Dynamics. Clearly, follicular growth and vitellogenesis is continuous, but it is convenient to categorize follicles into one of six stages of development. Stage 0 follicles are not detectably differentiated from other follicles in the germarium when viewed at 25X. Stage 1 follicles have no detectable yolk at 50X. Vitellogenesis is first seen in stage 2, and yolk may fill up to one-third of the follicle. Yolk occupies from one-third to two-thirds of the follicle length in stage 3. Stage 4 follicles have yolk that occupies more than two-thirds of their length. Pigmented eggs are scored as stage 5. Oviposition terminates stage 5, when the secondary series of follicles already are at stage 3 in 97% of the flies (Krafsur & Ernst 1983). Once oviposition has occurred, the parous fly is said to have ovaries in stage (5+3) = 8.

Stages of ovarian development were characterized further by measuring their lengths. A better scale for growth is afforded by volume rather than length, and volume was approximated by cubing the linear measure.

Estimating Survival Rates from Gonotrophic Data. Where a constant schedule of recruitment, vitellogenesis, and mortality applies (i.e., a stable age distribution) and sampling is representative of all ages, a survival distribution may be estimated from the proportion vitellogenic (*PV*), where p is the probability of surviving through one day and all flies at least two d old are vitellogenic:

$$PV = \frac{p^2 + p^3 + \ldots + p^\infty}{p^0 + p^1 + \ldots + p^\infty}$$

which simplifies to:

$$p = \sqrt{PV}.$$

Survival may be estimated also from the proportion parous, *PP*, where the median age at first oviposition is taken to be 3.5d:

$$PP = \frac{p^{3.5} + \ldots + p^\infty}{p^0 + \ldots + p^\infty}$$

$$p = \sqrt[3.5]{PP}$$

Survival also can be related to the proportion parous of only vitellogenic flies (*PPV*), thus:

$$PPV = \frac{p^{3.5} + p^4 + p^{4.5} + \ldots + p^\infty}{p^2 + p^{2.5} + \ldots + p^\infty}$$

$$p = \sqrt[1.5]{PPV}$$

Stage 2 follicles do not usually recur after the first gonotrophic cycle in horn flies and therefore need not be included in the parous category:

$$PPV' = \frac{p^{3.5} + \ldots}{p^{2.5} + \ldots} = p.$$

Statistical Methods. A variable to represent gonotrophic (physiological) age was constructed by the relationship,

$$\text{Gonotrophic age} = (5 \times \text{parity}) + \text{stage}.$$

The variables of thoracic length, $\log_e$ fluorescence value (LSPF), $\log_e$ gonotrophic age, $\log_e$ physiological age, and egg numbers were treated to analyses of variance by using SAS Proc GLM (SAS 1988). The age structure of a horn fly population varies continuously and many samples from a standing population therefore are neces-

Table 1. Ovarian development by stage and follicle volume among feral Iowa and laboratory-reared Texas horn flies

Stage	Iowa field-collected				Laboratory			
	n	Follicle length, mm[a]	Vol, mm^3	% Development	*n*	Follicle, mm[a]	Vol, mm^3	% Development
				Nullipars				
0	68	0.119 ± 0.041	0.002	0.08	52	0.04 ± 0.00	—	—
1	198	0.231 ± 0.131	0.012	0.61	60	0.19 ± 0.05	0.007	0.4
2	39	0.564 ± 0.140	0.179	8.95	3	0.67 ± 0.09	0.301	16.2
3	112	0.956 ± 0.180	1.158	58.00	20	1.05 ± 0.14	1.158	62.21
4	39	1.120 ± 0.116	1.405	70.00	29	1.20 ± 0.09	1.728	92.86
5	142	1.26 ± 0.065	2.000	100.00	29	1.23 ± 0.05	1.861	100.00
				Parous				
6	1	0.36 ± 0.076	0.045	2.25	—	—	—	—
7	12	0.60 ± 0.138	0.212	10.58	—	—	—	—
8	331	1.025 ± 0.151	1.077	53.83	18	1.01 ± 0.15	1.030	55.37
9	261	1.21 ± 0.106	1.772	88.56	19	1.20 ± 0.09	1.728	92.06
10	223	1.26 ± 0.068	2.000	100.00	20	1.23 ± 0.04	1.861	100.00

[a] Values are means ± SD.

sary to estimate the population's average survival rate (Caughley 1977, Birley 1984). However, successive observations on flies from the same herd (the same experimental unit) will be serially correlated and degrees of freedom will be exaggerated. Thus, an experimental design was used in which sampling days were nested in cattle herds, and herds were nested in years. Differences in annual means were tested by using the Type I mean squares for LOC(YR) as the error term.

Spectrofluorimetry. Procedures to estimate pterin concentrations generally follow Mail et al. (1983) and Lehane & Mail (1985). Fly heads were placed singly into 1.5-ml microcentrifuge tubes, and these were kept in the dark in a desiccating atmosphere for later pterin extraction. Heads were homogenized with a pestle in the centrifuge tubes to which 50 μl of sterile 50 m*M* pH 8.6 tris·HCl buffer had been added. A further 550 μl of buffer was added after homogenization. After 3 min centrifugation at 3,000 rpm, 450 μl of supernatant was pipetted into a numbered plastic 1.5-ml semimicro cuvette (Fisher Scientific Company, Chicago, Ill.), and a further 350 μl of buffer added. Homogenates were kept in the dark.

Fluorimetry was performed on a SLM6000 (SLM Instruments, Urbana, Ill.) specrofluorimeter. Excitation was set at 365 nm and emission at 450 nm. Separate wavelength scans of pterin standards (2-amino-4-pteridinol, Aldrich Chemical Company, Milwaukee, Wis.) and extracts from fly heads treated in the manner just described were identical, and emissions were maximum at 450 nm. Standards consisting of 5 × 10E-7 pterin (g/liter) in 50 m*M* pH 8.6 buffer gave relative fluorescence readings of 1,000, and these were used to calibrate the specrofluorimeter settings on each occasion. Fluorescence was recorded 10 s after initial excitation of the sample.

Results

Ovarian Growth Rates. Categories of ovarian development and corresponding linear and cubic measures are set forth in Table 1 for Iowa flies and laboratory-reared flies. The volumetric approximations afford estimates of percentage development—i.e., among stage 3 nullipars, development averaged 58% of completion. Most flies that recently oviposited had follicles ≈54% developed (i.e., stage 8, Table 1).

Stage 0 follicle sizes differed between field and laboratory flies. This was a sampling artifact. The laboratory flies were a mean 2.5 h old, having been collected from 0 to 5 h after eclosion. However, the feral Iowa flies were representative of all chronological ages that had stage 0 follicles.

Schedule of Oviposition. Data on mean body and follicle sizes, relative pterin concentrations (SPF), and reproductive indices for laboratory-reared horn flies of known ages are provided in Table 2. A weak ($r = 0.09$) but statistically significant ($P < 0.0001$) relationship was found between the number of ovarioles (Y) and length of the mesonotum, X ($Y = 8.58 + 6.93X$). Head widths did not correlate with ovariole numbers as well as did mesonotal lengths, so body sizes are hereinafter expressed as mesonotum lengths. There was no obvious relationship between body size and survivorship in the laboratory flies, as evident by the constancy of size with increasing age. Vitellogenesis was not observed among 24-h-old females. But once begun, vitellogenesis was rapid, and by 48 h, 13.5% of the flies were gravid and only 3% remained previtellogenic (stages 0, 1). Thus, about 24 h was necessary for the follicles to complete vitellogenesis. The first parous flies were found on day 3. These data are consistent with observations of Beadles et al. (1977) and Kuramochi & Nishijima (1984).

Table 2. Body sizes measured by mesonotal length, ovarian development as measured by ovary stage and proportions vitellogenic and parous, numbers of eggs, mean follicle lengths, and relative fluorescence among laboratory-reared horn flies

Age, d	n	Body size		Ovarian development			No. eggs	FL[c], mm	SPF[d] ± SD
		Mesonotal length, mm	CV	Mean ovary stage	VIT[a]	PP[b]			
0.1	52	1.34	4.5	0	0	0	—	0.04	20.4 ± 9.68
1	62	1.32	7.1	1.0	0	0	—	0.19	45.2 ± 21.77
2	37	1.37	8.9	3.5	0.97	0	18.4	1.06	66.7 ± 12.65
3	47	1.34	8.2	4.2	0.96	0.35	18.2	1.16	83.2 ± 14.63
5	30	1.34	6.1	4.27	1.0	1.0	18.6	1.20	135.7 ± 27.01
7	20	1.31	6.7	4.05	1.0	0.95	17.2	1.15	133.0 ± 22.97
9	11	1.33	6.2	4.0	1.0	1.0	17.9	1.06	165.9 ± 69.04
11	3	1.36	2.9	4.33	1.0	0.67	20.7	1.24	164.7 ± 47.37
13	3	1.36	5.9	4.0	1.0	1.0	22.0	1.18	234.3 ± 29.50
15	3	1.32	6.6	3.67	1.0	1.0	—	1.08	180.7 ± 51.54

[a] Proportion vitellogenic.
[b] Proportion parous.
[c] Follicle length.
[d] Relative fluorescence.

All laboratory flies oviposited by day 5, and the average stage of vitellogenesis was 4.3, subgravid (Table 2). The penultimate follicles of gravid flies were most frequently in stages 3 or 4 and had completed 58 to 70% of egg development (Table 1). It therefore seems probable that most horn flies at least 5 d old would have eggs to lay every day.

The number of functional ovarioles did not change with advancing fly age, suggesting that fecundity was invariant with increasing age.

Predicting Age with Pterin Concentrations. Least-squares regression of the fluorimetric readings on age (in days) of the laboratory flies (Table 2) provide the means to estimate chronological ages of field-collected flies. The equations relating SPF to age were:

males (n = 254):

$$AGE = 0.060(SPF) - 1.446,\ R^2 = 0.74,$$

females (n = 208):

$$AGE = 0.050(SPF) - 0.815,\ R^2 = 0.70.$$

SEs of the intercepts were: males, 0.347; females, 0.233; SEs of the slope were: males, 0.002; females, 0.002.

Gonotrophic Classes Among Iowa Horn Flies. The 11 gonotrophic classes recognized are shown in Table 3. We did not accurately discriminate multiparous from uniparous flies, so all parous flies are included in age groups 6–10.

The previtellogenic classes made up 18.6% of the total flies but 44% of the nulliparous flies. Stage 2 was brief, comprising 6.5% of the first gonotrophic cycle flies. Stage 3 accounted for 18.5% and stage 4 accounted for 6.4% of the nullipars; 23.5% of nulliparous flies were gravid. This large fraction of gravid flies suggests that there were oviposition delays. Recruitment and oviposition occur constantly in each horn fly population, so the proportions in each stage can be converted to relative durations, assuming no sampling bias with respect to age (Krafsur & Ernst 1983, 1986). The previtellogenic period takes about 57.5% of the time required to become gravid; stage 2 only 8.5%; stage 3, 18.5%; and stage 4, a brief 8.4% of the time.

The virtual absence of previtellogenic, parous flies (stage 6) and the low frequency of flies with stage 7 follicles are consistent with the observation that gonotrophic concordance does not occur in horn flies (Detinova 1962); instead, vitellogenesis proceeds simultaneously in two or three successive follicles, each in a serially less advanced stage of development. Thus, secondary follicles in horn flies are typically 58% developed when the primary follicles are mature.

Table 3. Distribution of physiological age classes of horn flies in Iowa

Age class	1988		1989	
	n	% In class	n	% In class
		Nulliparous		
0	18	6.4	50	15.5
1	91	32.3	107	33.1
2	18	6.4	21	6.5
3	43	15.3	69	21.4
4	22	7.8	17	5.3
5	83	29.5	59	18.3
Σ Nullipars	275	41.2	323	42.3
		Parous		
6	0	0	1	0.2
7	6	1.6	6	1.4
8	129	33.4	205	46.6
9	122	31.6	140	31.8
10	135	35.0	88	20.0
Σ Parous	392	58.7	440	57.7
Total	667	100.0	763	100.0

χ^2 = 49.66, df = 9, P = 10E-7.
Between year comparisons:
χ^2 (proportions previtellogenic) = 4.21, df = 1, P = 0.04.
χ^2 (proportions parous) = 0.178, df = 1, P = 0.66.
χ^2 (proportions gravid) = 26.96, df = 1, P = 0.0001.

Table 4. Analyses of variance of horn fly pterine concentrations (SPF), physiological ages, thoracic lengths, and egg numbers

Source	Log SPF				Log physiologic age				Thoracic length, mm				No. eggs			
	df	SS	F	P	df	SS	F	P	df	SS	F	P	df	SS	F	P
Yr	1	24.50	12.46	0.005	1	4.05	1.49	0.25	1	0.113	1.23	0.29	1	59.57	1.92	0.20
Loc(yr)	10	16.40	4.34	0.0001	9	11.76	3.35	0.005	10	0.436	2.63	0.004	9	176.10	2.11	0.03
Date	75	167.96	5.92	0.0001	71	119.06	4.30	0.0001	73	3.55	2.93	0.0001	66	1,461.25	2.39	0.0001
Loc*date(yr)	23	30.73	3.53	0.0001	23	28.77	3.21	0.0001	23	0.47	1.23	0.204	22	230.90	1.13	0.307
Error	1,413	534.60	—	—	1,320	514.76	—	—	1,336	22.16	—	—	577	5,350.28	—	—
Total	1,522	783.81	—	—	1,425	701.10	—	—	1,443	27.39	—	—	676	7,448.05	—	—

Previtellogenic flies occurred, with a single exception, in the first gonotrophic cycle, and stage 2 was detected infrequently in the second and subsequent cycles (Table 3). Semigravid flies (stage 4 or 9 follicles) seemed more likely to be judged parous than flies with ovaries in stage 3 or 8. Thus, there was bias in assigning a gonotrophic cycle even to developmental stages that recur in each cycle.

The proportions parous were homogeneous among years, but the relative proportions previtellogenic and gravid differed significantly (Table 3). The differences in proportions previtellogenic may have been an artifact of sampling. The overall mean previtellogenic was 18.6%, and the mean gravid was 25%. The proportions termed "gravid" differed between years because we were inconsistent in assigning flies to the gravid categories that had laid only a portion of their mature eggs.

Analyses of Variance. Analysis demonstrated statistically significant differences in egg numbers among locations and dates, but not years (Table 4). Fecundity varied directly with body size as measured by thoracic length, confirming the laboratory work. Body size also varied significantly among locations and dates. Significant differences were detected in gonotrophic ages among locations and years, although only ≈2.3% of the total variance was accounted for by years and locations (Table 4). A similar result was obtained with analysis of the pterin concentrations; here, 5.2% of the variance was "explained" by years and locations. However, most variation was accounted for by dates of sampling, because age structure of fly populations changes continuously.

Correlations of Chronological Ages with Gonotrophic Ages. Pterin fluorescence showed that vitellogenesis among wild flies (Table 5) proceeded as rapidly as among the laboratory flies. The frequency of "late" previtellogenics was only 2.1% among flies of chronological ages at least 2 d old, and 1% among 3-d-olds.

A proportionally larger fraction of "late" previtellogenic flies would result were there appreciable delays in their finding a host or in beginning vitellogenesis. These data, therefore, suggest that most females find a host and begin feeding on the day of eclosion. There were 8.9% vitellogenic day-0 flies, but this frequency falls within the expected error of the regression of age on SPF. The same reason explains the 11% 1-d-old gravids.

Proportions of gravid flies by age were homogeneous from day 3 onward (χ^2 = 10.87, df = 8, P = 0.21). Twenty-three percent of the estimated 2-d-old Iowa flies were gravid compared with 13.5% of the laboratory flies known to be 2 d old. Error in age prediction by using pterins accounted for the difference.

Calendar ages predicted by the pterin regression are compared with the age-grading results for the same horn flies (Table 5). Reproductive success, as measured by parity (Table 5), seemed to begin at day 0 and converged to 90% by day 6. The table confirms that it is difficult unambiguously to determine the gonotrophic cycle of gravids, particularly flies in which there were interrupted ovipositions. For example, most (69/93, 74%) nullipars estimated to be at least 4 d old were gravid flies. For this reason, reproductive success is shown also for nongravid flies (Table 5), where parity was 93% among flies at least 5 d old and 98% among flies at least 8 d old.

Survival Distributions. Application of the equations relating chronological age to fluorescence for the 1988 and 1989 data generated age distributions, which, when grouped into integer

Table 5. Cross-classification of field-collected horn fly female chronological ages with gonotrophic status

Predicted chronological age, d[a]	n	Gonotrophic status (stage)[b]					
		Previt (0,1)	Vit null (2,4)	Gravid null (5)	Vit parous (6–9)	Gravid parous (10)	Proportion parous (6–10/total)
0	225	205	14	3	3	0	0.013
1	145	39	84	12	16	4	0.138
2	180	13	62	29	63	13	0.422
3	175	4	21	29	98	23	0.691
4	190	2	11	28	109	40	0.784
5	158	2	2	18	98	38	0.860
6	105	0	5	9	52	39	0.870
7	85	0	1	6	57	21	0.920
8	48	0	0	0	35	13	1.00
9	37	1	0	3	24	9	0.973
10	24	0	0	3	14	8	0.875
≥11	54	0	0	2	37	15	0.963

[a] Estimated by pterin fluorescence.
[b] Established by dissection.

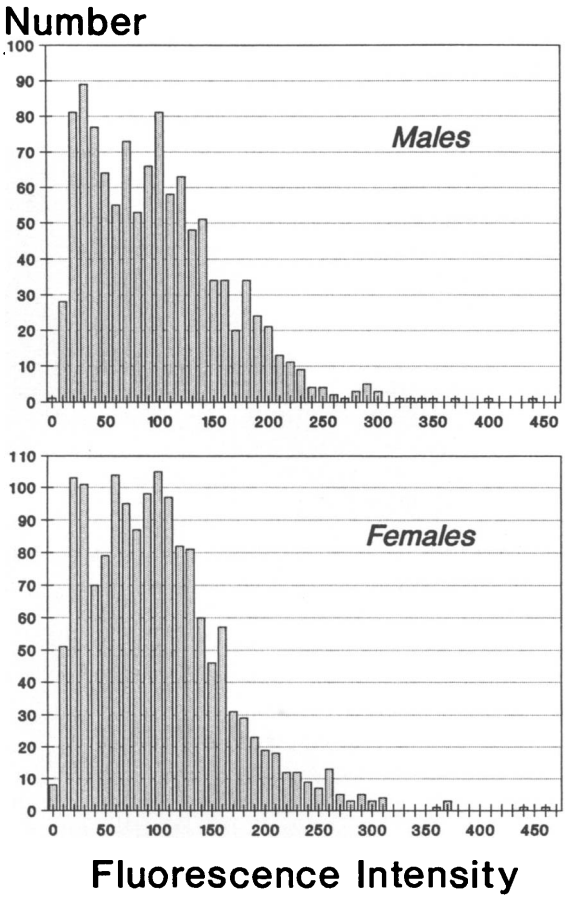

Fig. 1. Frequency distribution of pterin fluorescence intensities among field-collected horn flies by sex. 1,117 males and 1,523 females.

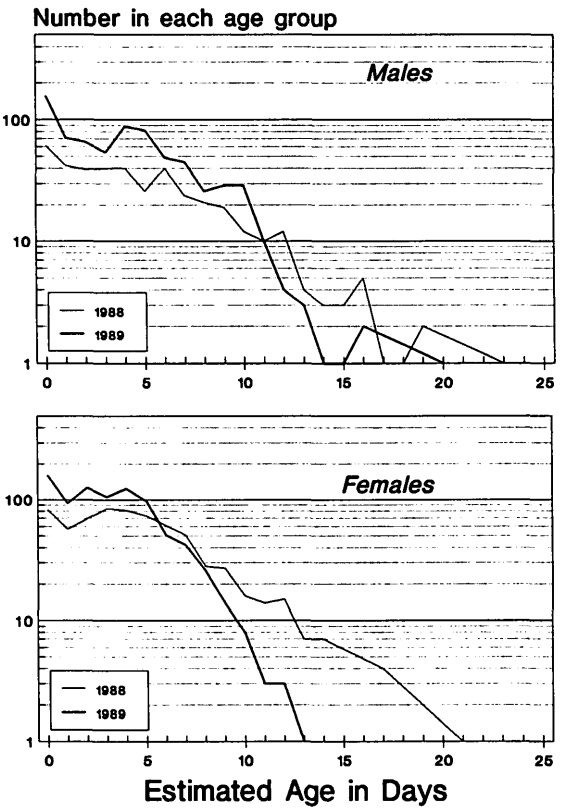

Fig. 2. Estimated survival distributions of horn flies calculated from pterin fluorescence by year and sex.

ages (Fig. 2), yield vertical life tables (Southwood 1977). The fluorescence distributions of Iowa horn flies were skewed to the right (Fig. 1), as expected on the assumptions that generations overlap continuously and that survival is exponentially distributed. The logarithmically-transformed frequency distributions (Fig. 2) suggest that survival rates were greater in 1988 than in 1989. Taking arithmetic means of the distributions showed that the average female was 5.1 d old in 1988 and 3.7 d in 1989; the average male was 5.4 d old in 1988 and 4.3 d in 1989.

The slope of regression of $\log_e$ numbers on chronological age estimates a mortality rate m averaged over all age groups and populations (Table 6). Rate m includes losses by emigration, but we can assume that this is balanced by immigration. Application of this simple exponential model assumes that the mortality rate was constant over all ages. Survival rates differed significantly between years both in males and females; we could find no obvious reason for this difference. If, nevertheless, data over years were combined and tested for differences in slope (mortality) between sexes, no statistically significant difference emerged (Table 6).

The constants for daily survival were calculated by year and sex (Table 7). The residuals were not randomly distributed with respect to age but were negative for ages 0–2 d and positive for ages 4–12 d. These departures of observed ages from the expected were small in magnitude,

Table 6. Numbers of flies alive each day regressed on estimated integer age using pterin method of predicting age

Yr	No. age groups	Origin ± SE	Slope ± SE	R^2
		Males		
1988	22	4.168 ± 0.141	−0.163 ± 0.011	0.92
1989	18	5.107 ± 0.232	−0.251 ± 0.023	0.88
Mean	22	5.423 ± 0.188	−0.224 ± 0.014	0.92
		Females		
1988	18	4.851 ± 0.126	−0.189 ± 0.011	0.95
1989	14	5.622 ± 0.244	−0.339 ± 0.032	0.90
Mean	18	5.849 ± 0.140	−0.245 ± 0.013	0.96

Tests for homogeneity of slopes:
Males by year: $F = 14.19$; df = 1; 36; $P = 0.0006$.
Females by year: $F = 25.47$; df = 1, 28; $P = 0.0001$.
By sex: $F = 1.09$; df = 1, 46; $P = 0.091$.

Table 7. Demographic statistics derived from horn fly survival distributions estimated by pterin fluorescence, by year

Demographic statistic	1988		1989	
	♂♂	♀♀	♂♂	♀♀
Daily survival (p)	0.837	0.811	0.749	0.661
Expectation of life in days	5.62	4.77	3.46	2.42
Mean lifetime ovipositions	—	2.82	—	0.85
Mean no. eggs/lifetime	—	25.96	—	7.84
Critical egg-to-adult survival rate	—	0.04	—	0.13

and the exponential model provided better fits than Weibull and gamma models (data not shown).

Demographic Statistics. The survival rate of females can be used to calculate their mean expectation of life and mean lifetime fecundity at eclosion (Table 7). The mean expectation of life of adults at eclosion is given by the expression, $-\log_e p^{-1}$ (Cook 1971, Rogers & Randolf 1984).

Summing the proportions surviving, p^n, for ages n of 0 to 22 d multiplied by the age-specific estimate of reproductive success (the proportions parous, Table 3) gave an estimate of the mean number of ovipositions per lifetime. Multiplying this value by the mean number of eggs per clutch, 18.4, gave the mean number of eggs per lifetime. Another, quicker method is provided by the expression,

$$p^{3.5}/(1 - p)$$

where the median age at first oviposition is taken to be 3.5 d and further ovipositions occur daily thereafter.

To estimate net reproduction rates (R_0) from the foregoing data, we assumed a sex ratio of unity and let S be the mean egg to adult survival rate. Then $R_0 = S \times$ proportion female $\times [(p^{3.5})/(1 - p)] \times$ mean eggs per clutch (Table 7). An average female will replace herself with only one reproducing daughter in a stationary population where $R_0 = 1$. The values of S that satisfy the expression $R_0 = 1$ are set forth in Table 7.

Analysis of Ovarian Data. Estimates based on the proportions vitellogenic gave the highest values of mean daily survival, p (Table 8). The proportion parous estimated p to be ≈0.85. A lesser estimate of p equals ≈0.80 resulted when previtellogenic flies were deleted. Yet a lesser estimate of p was obtained when flies with only ovaries in stages 3–5 and 8–10 were considered; here $PPV' = p = 0.73$ (Table 8). Also, stage 3 follicles occurred at approximately daily intervals giving a mean $p = 0.75$. These last two estimates were homogeneous ($\chi^2 = 0.235$, df = 1, $P = 0.63$). Remarkably, there were no between-year differences in p.

Table 8. Estimates of female horn fly survival (p) based on gonotrophic age distributions

Method	1988		1989	
	Proportion[a]	p	Proportion[a]	p
Vitellogenic (*PV*)	558/667	0.915	606/763	0.891
Propns. parous (*PP*)	392/667	0.859	440/763	0.855
Parous of vits. (*PPV*)	392/558	0.790	440/606	0.808
PPV, less stages 2, 7	386/534	0.723	433/578	0.749
PP, stages 3, 8 only	129/172	0.750	205/274	0.748

[a] Gonotrophic proportions were taken from Table 3.

The estimates of survival that incorporated flies with previtellogenic ovaries were significantly greater than estimates based on more advanced gonotrophic development. If sampling was indeed representative of all ages, the foregoing calculations suggest that survival rates declined with increasing age. If sampling was not representative, it would seem that the youngest age groups were less likely to be sampled than older age groups.

Discussion

Measurement of pterin concentrations allows, for the first time, estimation of chronological ages of insects without the problems that mark, release, and recapture experiments entail. Pterins are deposited at rates that vary directly with the product of time and temperature. Given the thermal history of sampled flies, their chronological ages can be estimated. For horn flies, a thermal history at the geographic locations where flies were sampled does not seem necessary because of behavioral thermoregulation on the host (Morgan 1964, Kuramochi 1989). It would be useful to verify this assumption by mark, release, and recapture experiments at different times over a full season.

Combining two independent age-grading methods allows estimates of female reproductive success to be correlated with chronological age. Oviposition delays can be assessed, and more accurate vertical life tables can be constructed than on the basis of ovarian dynamics alone. The present field data suggest that a proportion (108/701, 15%) of horn flies experienced oviposition delays. But a nearly equal number (100) and proportion (100/550, 18%) seem to have oviposited early. However, the flies of known ages from laboratory provide no evidence of ovipositing before day 3. It is easy to suppose that flies in the field might suffer delays compared with flies confined to a laboratory, but more rapid ovarian development times in the field seems very improbable. Most of the "early" and "late" reproducing horn flies, in fact, fell within the confidence interval about the pterin concentration–age regression line. Thus, no net oviposition delays were accrued in field samples, and we detected only

small numbers of parous flies in the early stages of vitellogenesis. Further, vitellogenesis continues in flies that have deposited only a portion of their mature eggs. Dysfunctional ovarioles are rare in horn flies (Krafsur & Ernst 1983). It seems, therefore, that horn fly females continuously oviposit until they die.

Proportionally few females at least 4 d old failed to oviposit, and these old nullipars (90) were almost compensated by the numbers of parous flies <3 d old (99). Horn flies thus show a high frequency of reproductive success. Moreover, most females seemed to do so at the maximum sustainable rate as is suggested by the low frequency of parous flies with stage 1 or 2 ovaries.

Horn fly age distributions as measured by SPF differed between 1988 and 1989, whereas physiological ages did not differ significantly. Log SPF provides the more sensitive index of age because it forms a continuous distribution that is approximately normal, whereas the index of physiological age varies from 0 to 10 and is distributed bimodally, even after transformation. However, both indices show that most of the attributable variation corresponds to dates of sampling. The principal reason that a stable age distribution was not obtained was because of the variable recruitment of new adults into standing populations, and age distributions are not stable. In fact, most variation in age-related indices was unexplained "error" variance.

The use of ovarian dynamics to construct a vertical life table is made difficult by uncertainties in representativeness of sampling and by bias in assignment of flies to the correct gonotrophic cycle. Thus, the earlier estimates of horn fly survival (Krafsur & Ernst 1983, 1986) were too high. But it seems that an acceptable estimate of a mean survival rate can be obtained by taking the parous proportion of flies in ovarian stages 2 and above. For the Iowa female horn flies, we estimated mean daily survival p to vary from 0.66 to 0.81 by using the pterin method. The gonotrophic method provided estimates of p that varied from 0.72 to 0.91. A progressive increase in daily mortality is suggested by using progressively older flies in the gonotrophic method. This result could be from age-related bias in sampling, where the youngest flies are less subject to capture than older flies. But sampling does not explain the difference in SPF between years.

Caution needs to be attached to estimates of survivorship (i.e., p) based on one or two points in the age distribution, as is done when using gonotrophic ages. Even when linear relationships can be assumed legitimately, small changes in slope (i.e. the survival rate) give large changes in the area under the distribution of survivorship on age.

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